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Genetic analysis of resistance to ear rot and mycotoxin contamination caused by *Fusarium graminearum* in European maize

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1. General introduction

Maize (*Zea mays* L.) is a major crop for food and feed production in Europe. In 2009, 13 million ha of arable land were cultivated with maize in Europe (FAOSTAT, 2011). Maize growers have to cope with a range of different diseases, the prevalence of which depends mainly on the region under consideration. In general, all parts of the maize plant are susceptible to certain diseases (White and Carson, 1999). As a consequence, much attention is paid to diseases of the maize ear, because high grain yields and qualities can be ensured only by healthy ears and kernels. Among the various ear rot diseases of maize, the most prevalent in Europe are those caused by the genus *Fusarium*.

Epidemiology of *Fusarium graminearum*

The genus *Fusarium* includes a range of toxigenic fungi, which give cause for concern in many maize growing regions in the world. Occurrence of different *Fusarium* species on maize in Europe is very much dependent on the climatic conditions (Logrieco et al., 2002). In the hot and dry areas of Southern Europe, *F. verticillioides* (Sacc.) Nirenberg and *F. proliferatum* (Matsushima) Nirenberg are very widespread. In Northern and Central Europe, the prevalent *Fusarium* species on maize is *F. graminearum* Schwabe (teleomorph *Gibberella zeae* (Schwein.) Petch). Infections with this pathogen are frequent in years characterized by low temperature and frequent rainfalls during summer and autumn. For instance, *F. graminearum* was among the predominant *Fusarium* species on naturally infected maize ears in surveys in Germany in 2006 and in 2007 (Goertz et al., 2010) and in Switzerland in 2005 and 2006 (Dorn et al., 2009).

Main sources of inoculum for new infections are colonized plant residues. Infections mainly take place through sexually formed ascospores as well as through asexually formed macroconidia. Ascospores are spread by wind, whereas macroconidia are spread by splashing and wind driven rain (Sutton, 1982). *F. graminearum* is able to colonize almost all plant tissues. Owing to its teleomorphic name and to the infected plant tissue, the corresponding diseases in maize are called Gibberella stalk rot and Gibberella ear rot (GER). The latter is sometimes also referred to as red ear rot. The primary mode of

GER infection is via the maize silks (= styles) mediated through germination of spores on the silks and followed by growth of hyphae, which penetrate the cob and immature maize kernels. Mechanical ear wounds caused for example by birds, insects or hail, can promote infections, too. However, wound infection is considered less important compared to silk infection (Sutton, 1982; Munkvold, 2003a).

Typically for GER is a red or pink mold that usually starts at the ear tip and spreads downwards (Figure 1). Early infections can lead to completely diseased ears covered with reddish mycelium with husks tightly adhering onto them (Payne, 1999).

Figure 1 Severe symptoms of Gibberella ear rot on artificially infected maize ears.

Consequences of Gibberella ear rot

GER can lead to yield losses (Vigier et al., 2001), yet far more important under Central European growing conditions is contamination of the maize grains with mycotoxins. These fungal metabolites reduce the quality of harvested grains and consumption can lead to serious consequences for the health of humans and animals. Among the various mycotoxins produced by *F. graminearum*, the most prevalent are deoxynivalenol (DON) and zearalenone (ZEA). DON belongs to the trichothecenes, a group of immunosupressive mycotoxins associated with toxicoses of mammals. DON is considered as the most common trichothecene on cereals and as a potential aggressiveness factor of the fungus (Glenn, 2007). Consumption of maize contaminated by ZEA is problematic especially for women due to its mycoestrogenic properties. In pigs, which are the most sensitive farm animals, ZEA causes prolonged estrus intervals, stillbirth and other fertility disorders (Fink-Gremmels and Malekinejad, 2007). Especially in maize, ZEA has been encountered at very high concentrations (Bottalico, 1998). Further, contaminations lead to economic losses due to reduced feed intakes and performances of animals, as well as rejections in the market (Wu, 2007). The European Union notified maximum levels for certain food contaminants, including DON and ZEA, to protect public health. The limits in unprocessed maize intended for use as food are 1.75 mg kg^{-1} of DON and 0.35 mg kg^{-1} of ZEA (European Commission, 2007). Furthermore, for animal grain feed, guidance values are 0.9-12 mg kg^{-1} for DON and 0.1-3 mg kg^{-1} for ZEA, depending on the animal and its age (European Commission, 2006a). Surveys from the last ten years show that DON concentration in grain maize varies tremendously depending on the variety, location and year. Furthermore, the concentration in many samples is regularly beyond the legal threshold level set by the European Commission (Eder et al., 2011).

Prevention of mycotoxin accumulation

So far, there is no efficient fungicidal control of ear rots in maize available. Therefore, certain risk factors should be taken into account, to reduce mycotoxin contaminations, following good agricultural practices, e.g., choice of varieties, crop rotation, crop planning,

soil management, harvesting and post-harvest measures (European Commission, 2006b). But cultural practices have only limited effects on infection and mycotoxin accumulation, whereas post-harvest measures can only prevent further development of mycotoxins. Therefore, genetic resistance to ear rots and associated mycotoxins has the greatest potential (Munkvold, 2003b). The use of transgenic varieties, which carry a *Bacillus thuringiensis* (*Bt*) derived gene encoding for a insecticide toxin, has been proposed for prevention of mycotoxin contamination (Munkvold et al., 1997). In field experiments with European corn borer infestation, *Bt* varieties showed lower mycotoxin contamination than their isogenic counterparts (Magg et al., 2002; Papst et al., 2005). However, due to the prevalence of silk infection and the lacking acceptance of transgenic plants in Europe, genetic improvement of maize by means of classical resistance breeding seems most promising.

Genetic improvement of resistance to Gibberella ear rot and mycotoxin accumulation

Resistance to Gibberella ear rot and mycotoxin accumulation is inherited quantitatively (Chungu et al., 1996; Ali et al., 2005; Bolduan et al., 2009). Since GER epidemics are sporadic and disease severity is strongly influenced by the environment, artificial inoculation is usually performed to assess the resistance of the germplasm. Artificial inoculation enhances infections and overcomes the variability of certain years, when natural infection levels are too low to identify genotypic differences (Reid et al., 1996a).

Genotypic variation for resistance to GER and mycotoxin accumulation has been reported for European (Bolduan et al., 2009; Löffler et al., 2010) and South- and North-American maize germplasm (Presello et al., 2004; Reid et al., 1993; Reid et al., 1996b) and heritabilities were reported to be moderate to high. DON concentration and GER severity showed a strong positive relationship in various maize materials. In contrast, the relationship between ZEA concentration and GER severity is less clear, as correlations were reported to vary from not significant to strong (Atlin et al., 1983; Cullen et al., 1983; Bolduan et al., 2009; Löffler et al., 2010; Reid et al., 1996b). Depending on the strength of these correlations, phenotypic selection may be conducted either directly by mycotoxin analyses or indirectly by ear rot evaluations.

Phenotypic selection for increased resistance represents a difficult and time consuming process because of labor-intensive field experiments and expensive mycotoxin analyses. An efficient alternative to classical phenotypic selection might be provided by marker-assisted selection (MAS). In quantitative genetics, MAS describes a method of integrating molecular genetics with artificial selection (Lande and Thompson, 1990). MAS has been proposed by a number of authors, who conducted quantitative trait loci (QTL) mapping studies on the resistance of maize to ear rots caused by different *Fusarium* species. Ali et al. (2005) found several QTL for resistance to GER after artificial inoculation in a Canadian recombinant inbred line population. However, they also noted a strong influence of the environment on disease resistance. QTL mapping experiments on resistance to Fusarium ear rot (FER), which is mainly caused by *F. verticillioides* and *F. proliferatum*, showed a relative complex inheritance (Ding et al., 2008; Pérez-Brito et al., 2001; Robertson-Hoyt et al., 2006). Many of the detected QTL could only explain moderate or small proportions of the total phenotypic variance and in some cases epistasis seemed to play an important role. Robertson-Hoyt et al. (2006) found QTL having effects on both FER severity and fumonisin content, which provides an explanation for the strong genetic correlation between these traits at the molecular level. In a recent meta analysis of published QTL studies, several meta QTL influencing the resistance to three different ear rot diseases of maize (GER, FER, Aspergillus ear rot) were identified (Xiang et al., 2010). This indicated the presence of genomic regions, which confer unspecific resistance to different ear rots of maize.

Maize varieties grown in Europe are nearly exclusively developed in hybrid breeding programs. During the selection process, a large number of inbreds or doubled haploid (DH) lines is usually tested for their *per se* performance and for their testcross performance. For setting up a breeding program, it is essential to have information on heritabilities of and genotypic correlations between line and testcross performances as well as on the relevance of different modes of gene action. Depending on the magnitude of correlations and heritabilities, selection concentrates either on the one or the other type of performance. Although additive gene action is considered to be of primary importance for resistance to GER (Gendloff et al., 1986; Chungu et al., 1996), the correlations between lines and their testcross progenies have been reported to be low (Bolduan et al., 2010; Löffler et al., 2011). Therefore, prediction of hybrid performance based on parent line performance is quite difficult. This might indicate presence of non-additive gene action in

hybrids and/or a consequence of inbreeding depression of lines contrasted to better vigor of hybrids.

In applied breeding programs, selection is always conducted for several traits simultaneously, e.g., by using selection indices. Therefore, apart from correlations between and heritabilities of line and testcross performances, it is also necessary to consider correlations between the traits under selection. Generally, simultaneous improvement of undesirably correlated traits is difficult, whereas desirable correlations accelerate the selection process. Robertson-Hoyt et al. (2007) reported a weak relationship between the resistance to FER of lines and agronomic performance of their testcrosses. However, little is known about how selection for increased resistance to GER and mycotoxin contamination affects the expression of agronomically important traits such as grain yield.

Objectives of this study

The objectives of this study were to

(1) estimate quantitative-genetic parameters for GER severity and mycotoxin concentration in connected populations of doubled haploid (DH) lines (Publication 1 and 2),
(2) map QTL for GER resistance and reduced mycotoxin contamination in these populations (Publication 1 and 2),
(3) examine the congruency of QTL in these populations (Publication 2),
(4) evaluate the prospects of using MAS to breed for GER resistance and reduced mycotoxin contamination (Publication 1 and 2),
(5) estimate the genotypic correlation between the resistance of DH lines *per se* and the resistance of their testcrosses (Publication 3),
(6) evaluate the influence of selection for increased resistance on agronomic performance of hybrids (Publication 3) and
(7) examine the relevance of different modes of gene action involved in the expression of the resistance in flint maize (Publication 4).

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2. Publication 1: Colocalization of QTL for Gibberella ear rot resistance and low mycotoxin contamination in early European maize

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Crop Science, 2011 51:1935-1945

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Abstract

Fusarium graminearum Schwabe causes Gibberella ear rot (GER) of maize (*Zea mays* L.), an important disease in Europe, which reduces grain yield and leads to contamination with the mycotoxins deoxynivalenol (DON) and zearalenone (ZEA). The objectives of this study were to (i) estimate quantitative-genetic parameters for GER severity, DON and ZEA contaminations, (ii) map quantitative trait loci (QTL) for GER resistance, reduced DON and ZEA contaminations, and (iii) examine the prospects of marker-assisted selection (MAS) for these traits. The plant material, comprising 150 doubled haploid lines from a cross of two maize inbreds, was evaluated under artificial inoculation with *F. graminearum* across 2 locations in 2009 and 2010. Heritabilities on an entry mean basis across environments were moderately high (0.65-0.77) and QTL analyses identified four to six QTL for these traits. Identification of colocalized QTL in bins 1.11 and 2.04 with large effects, together explaining 29 to 35% of the total genotypic variance, suggested the presence of identical QTL with pleiotropic effects. This was supported by strong genotypic correlations among these traits (0.89-0.95). Due to the lower costs of genotyping compared to phenotyping, the study showed that breeding for GER resistance and reduced mycotoxin contamination, could be accelerated by adopting MAS. Application of MAS would be most efficient in off-season nurseries and in combination with phenotypic selection during the crop season.

3. Publication 2: Comparative QTL mapping for Gibberella ear rot resistance and reduced deoxynivalenol contamination across connected maize populations

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Abstract

Fusarium graminearum Schwabe causes Gibberella ear rot (GER) of maize (*Zea mays* L.) leading to yield losses and contamination with the immunsuppresisve mycotoxin deoxynivalenol (DON). To increase marker-assisted selection (MAS) efficiency, identification of QTL, which are effective across different genetic backgrounds, is necessary. The objectives of this study were to (i) map QTL for resistance to GER and reduced DON contamination, (ii) examine the congruency of QTL across four connected populations, and (iii) draw conclusions about the prospects of MAS. The populations under study comprised doubled haploid (DH) lines derived from crosses of four flint inbreds. GER severity and DON concentration were recorded after artificial inoculation with spores of *F. graminearum*. The estimates of genotypic variances (σ_G^2) were significant and heritabilities were moderately high to high. The detected QTL together explained 21-49% of σ_G^2 for GER severity and 19-30% for DON concentration. Colocalized QTL for resistance to GER and reduced DON contamination were identified in each of the four mapping populations. In addition, QTL located at similar positions were detected across three populations in two chromosomal regions and across two populations in additional two regions. The results suggested a combination of classical phenotypic selection and MAS as a promising strategy in breeding maize for resistance to GER and reduced DON contamination.

4. Publication 3: Variation and covariation for Gibberella ear rot resistance and agronomic traits in testcrosses of doubled haploid maize lines

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Euphytica

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Abstract

Fusarium graminearum Schwabe causes Gibberella ear rot (GER) of maize, a disease, which leads to reduction of grain yield and contamination with deoxynivalenol (DON). DON is an important mycotoxin adversely affecting the health of humans and animals. The objectives of this study were to (i) analyze means and genotypic variances for line *per se* performance (LP) and testcross performance (TP) of doubled haploid (DH) lines for GER severity and DON concentration as well as for some agronomic traits, (ii) examine genotypic correlations among these traits, (iii) validate QTL for resistance detected in previous studies for LP and their effect on TP and (iv) investigate the relative efficiency of indirect selection (*RE*) for LP to improve TP. Testcross progenies of 94 DH lines originating from four flint populations were developed using a susceptible dent tester as pollinator. LP was evaluated at two locations in 2008 and 2009, while TP was evaluated at the same two locations in 2009 and 2010. Artificial inoculations using conidia spores of *F. graminearum* led to appreciable disease development in lines and testcrosses. Average GER severity and DON concentration in the testcrosses were lower than the theoretical mean mid-parent values of the tester and DH lines, indicating mid-parent heterosis for resistance. Genotypic variation for resistance was significant for LP and TP. Genotypic correlations between LP and TP for GER severity and DON concentration were low. Accordingly, resistance QTL for LP had no significant effects on TP. *RE* for resistance was low. Therefore, resources should be mainly allocated for the evaluation of testcrosses. Correlations of resistance to GER and DON contamination with important agronomic traits (grain yield under non-inoculated conditions, number of days to silking, plant height) were not significant. This indicated that selection for resistance and these agronomic traits can be carried out simultaneously.

5. Publication 4: Inheritance of resistance to Gibberella ear rot and deoxynivalenol contamination in five flint maize crosses

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Abstract

Fusarium graminearum causes Gibberella ear rot (GER) of maize leading to contamination of grains with deoxynivalenol (DON), a mycotoxin that reduces the quality of food and feed. A generation means analysis was conducted to estimate significance of different modes of gene action for resistance to GER and DON contamination in flint maize germplasm adapted to Central Europe. Five crosses of two resistant (UH006, UH007) and two susceptible (UH009, D152) flint inbred lines were developed and each cross comprised both parent lines (P1, P2) as well as the F_1 and F_2 generation and the first backcross to both parents (BC_1-P1, BC_1-P2). The entries were evaluated after artificial inoculation with *F. graminearum* at two locations in 2009 and 2010. The results indicated the prevalence of additive gene action. Significant dominance effects were found only in one cross for resistance to GER, but in four crosses for resistance to DON contamination. Due to the importance of additive gene action, the prospects look good to realize improvement of resistance and accumulation of more favorable gene combinations in future breeding lines of the flint germplasm group.

6. General discussion

Genetical properties of the doubled haploid lines populations under study

In the present work, five populations of doubled haploid (DH) maize lines (D152×UH006, D152×UH007, UH009×UH006, UH009×UH007 and UH007×UH006) derived from crosses of four flint inbred lines were studied for various genotypic and phenotypic characteristics. Population sizes were quite variable and ranged from 46 individuals in the smallest population (D152×UH006) to 227 individuals in the largest population (UH009×UH007). For QTL mapping experiments, it is reasonable to have mapping populations with at least 100 individuals, because this number is necessary to detect a QTL explaining 10% of the phenotypic variance with a power of 85% (Charcosset and Gallais, 1996). This population size is therefore often considered as lowest boundary. Accordingly, QTL analyses of the smallest population (D152×UH006) were not informative and for that reason not included in the publications preceding this general discussion. However, it is worthwhile to discuss all five populations together regarding their relatedness and their genetical properties.

Genetic linkage maps and levels of polymorphism

The marker ordering along the chromosomes of the five individual linkage maps was generally in good agreement with that of the IBM2 2008 Neighbors map (Lawrence et al., 2008) serving as a reference map. Depending on the population, the proportion of polymorphic markers relative to all markers ranged from 34% polymorphism (UH009×UH007) to 50% polymorphism (D152×UH006). Associations among the four parental lines based on modified Roger's distance estimates (Reif et al., 2005) using SSR marker data are illustrated in Figure 2, which shows that parents UH007 and UH009 are closer related compared for example to D152 with the others. In harmony with this, a similar dendrogram was obtained from the pedigree data.

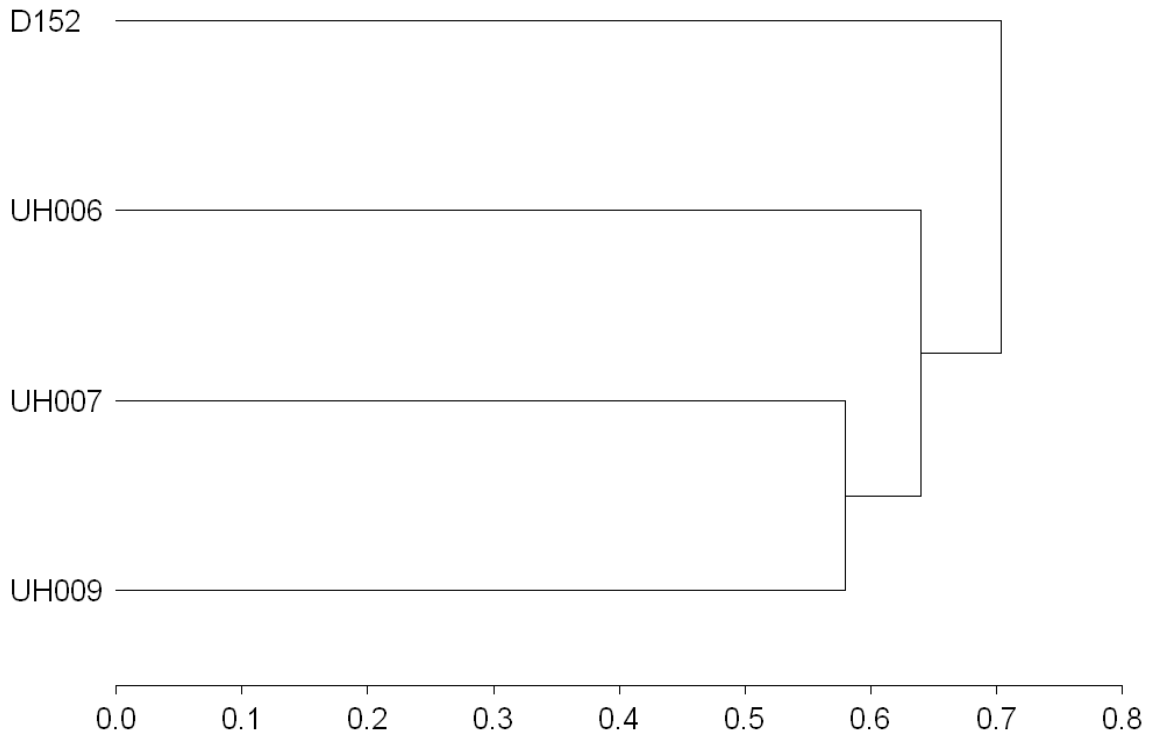

Figure 2 Dendrogram showing associations among the four parent lines (D152, UH006, UH007 and UH009) revealed by unweighted pair-group method using arithmetic averages cluster analysis based on modified Roger's distance (horizontal axis). Analyses were based on 307 shared SSR markers.

Moderate to low levels of polymorphism based on marker data among the parental lines indicated further that some chromosomal regions are identical by descent. This may be expected, because the parent lines are elite inbreds, which are adapted to the Central European climate and have common ancestors derived from French, German and Spanish flint germplasm in their pedigrees. In applied breeding programs, it is common to derive new lines from crosses of already adapted breeding lines. However, it is likely that this was responsible for the poorer marker coverage on certain chromosomes, the loose linkage between certain segments and representation of the ten maize chromosomes by eleven separate linkage groups in four of the mapping populations. Many markers especially on chromosome 7 were monomorphic among the parents and, thus, marker coverage on this chromosome was unsatisfying in all populations. This could indicate the presence of genes needed for superior agronomic performance and/or climatic adaptation on this chromosome, which have become fixed during selection for these traits.

Genome proportions and segregation distortion

The distributions of the genome proportions of UH006 and UH007 in the five populations were as expected for DH populations derived from the F_1 generation. The genome proportions were more or less normally distributed and had means ranging from 49 to 53% with standard deviations ranging from 8 to 13% depending on the population under consideration. However, for some markers, significant deviations from the expected Mendelian segregation ratio (segregation distortion) of 1:1 in DH lines were observed.

The proportion of mapped markers showing segregation distortion ranged from 12 to 27% ($P \leq 0.01$) and is within the range reported in other studies with maize (Lu et al., 2002; Ali et al., 2005; Zhang et al., 2006; McMullen et al., 2009). According to Zhang et al. (2010) segregation distortion is not supposed to have a significant impact on the estimation of QTL locations and effects. The reasons for segregation distortion, however, are not clear. The populations of DH lines under study were developed by pollinating F_1 plants of all five crosses with the pollen of an inducer line, followed by identification of haploid seeds and subsequent chromosome doubling of the haploid seedlings with colchicine treatment. There might be genetic factors, which enhance the haploid induction rate during the fertilization process, and/or the tolerance of seedlings to colchicine treatment and transplanting. These may lead to distorted segregation of genomic regions carrying such factors, but this is an area, where information is still lacking. Across all five populations, distorted markers were observed on all chromosomes, but certain chromosome regions were more affected than others. Interestingly, in the two populations having UH009 as a common parent (UH009×UH006, UH009×UH007), large proportions of chromosome 10 exhibited an under-representation of the UH009 genome. One explanation for this observation might be that UH009 possesses factors on this chromosome, which act against successful haploid induction or chromosome doubling. As a consequence, genotypes carrying the responsible alleles of UH009 may have got lost during DH development. In populations D152×UH007, UH009×UH006 and UH007×UH006, large proportions of chromosome 7 were distorted. In addition, some distorted regions from the present study were congruent with distorted regions, including those on chromosomes 7 and 10, described earlier in populations developed by selfing or random mating (Lu et al., 2002). This suggests that certain maize chromosomes are ubiquitously prone to segregation distortion. Further studies on gametophytic factors and

their impact on DH development could be worthwhile, because the use of DH lines has become common practice in maize breeding.

Genetics of resistance to Gibberella ear rot and mycotoxin contamination

In previous studies of Bolduan et al. (2009a) and in the present study, inbred lines UH006 and UH007 demonstrated their superiority in terms of resistance, whereas D152 and UH009 showed very high susceptibility. In the light of their response to infection with *F. graminearum*, the main purpose behind crossing these four lines was the development of DH populations showing large genotypic variation to conduct QTL mapping experiments, in other words to identify genomic regions responsible for increased resistance. Apart from this main objective, the DH lines developed from these recombinations represent potential new breeding lines and more than 25% of them have already been evaluated as testcrosses for agronomically important traits such as grain yield. As a consequence, some of these DH lines are meanwhile used as breeding lines within the maize breeding program of the Universität Hohenheim (personal communication, W. Schipprack, 2011).

Genotypic variation in DH lines and testcrosses

For the DH line populations, estimates of the genotypic variances (σ_G^2) and genotype-by-environment interaction variances (σ_{GE}^2) for GER severity, DON and ZEA concentration were significant in all instances (Martin et al., 2011; Martin et al., 2012a). Significance of these two parameters showed that (1) differences among DH lines for the resistance traits had genetic causes and (2) the DH lines at least partly responded differently depending on the environment. A common goal of plant breeders is selection of genotypes, which exhibit stable expression for the measured traits in many target environments. In regard of this, the present study clearly shows that selection decisions aiming at the improvement of resistance to GER and mycotoxin contamination need to be based on resistance screenings across multiple environments. The estimates of heritabilities (h^2) for the resistance traits were moderately high to high and varied in magnitude among the populations, namely

from 0.69 to 0.82 for GER severity, from 0.64 to 0.80 for DON concentration and for ZEA concentration the estimate was 0.65. In the present study, DH lines were evaluated in four environments, and thus, the prospects seem good that line resistance can be improved using a reasonable number of locations and years for resistance tests. The results on genotypic variances and heritabilities are in good agreement with earlier studies on GER, DON and ZEA (Reid et al., 1995; Chungu et al., 1996; Ali et al., 2005; Bolduan et al., 2009a; Löffler et al., 2010a).

Owing to the large genotypic variance for resistance, the populations may furthermore be described in terms of their usefulness for selecting superior individuals as defined by Schnell (1983). The usefulness of a population depends on its average performance, the square root of the heritability, the genotypic standard deviation and the applied selection intensity. In this regard, populations UH007×UH006 and UH009×UH006 were both similar superior to the other populations for GER resistance consistently across varying selection intensities. Thus, in these populations the chances are most promising to select superior transgressive segregants, i.e., progeny, which outperform the better parent due to accumulation of favorable QTL alleles from both parents. Indeed, some of these DH lines showed superior disease resistance, yet the differences to the better parent (UH006) were statistically not significant.

More important than *per se* performance of maize lines is their performance in hybrid combinations with genotypes from the opposite heterotic pool. Therefore, 94 DH lines from the four largest populations and their testcrosses with a moderately susceptible dent single cross tester were evaluated for GER severity and DON concentration (Martin et al., 2012b). For GER severity and DON concentration, the estimates of σ_G^2 of testcrosses within populations were five to three times smaller, respectively, compared to those of the corresponding DH lines. The estimates of σ_{GE}^2 of testcrosses within populations were in contrast to the corresponding DH lines not significant. Smaller genotypic variances in testcrosses were expected according to quantitative genetic theory (see e.g. Hallauer et al., 2010). Lack of significance of estimates of σ_{GE}^2 of testcrosses within populations indicated that resistance was expressed relatively stable across the four test environments. In contrast, significant genotype-by-environment interactions were reported in previous studies on testcrosses (Bolduan et al., 2010; Löffler et al., 2011). However, a significant population-by-environment interaction effect suggested a different response in the test environments depending on the source population. The estimates of h^2 for GER severity

and DON concentration of the testcrosses were only moderate and lower compared to those of the corresponding DH lines. This suggests that selection will be effective in both groups, but progress will be slower in testcrosses compared to lines assuming identical selection intensities.

Colocalization of QTL and genotypic correlations

Estimates of the genotypic correlation (r_g) between visually rated GER severity and mycotoxin concentrations were high ($r_g \geq 0.9$) in DH lines as well as in testcrosses (Martin et al., 2011; Martin et al., 2012a, b). This close relationship is in harmony with the majority of previous studies and indicates that mycotoxin contamination is a consequence of fungal growth (Reid et al., 1996; Bolduan et al., 2009a; Czembor and Ochodzki, 2009; Löffler et al., 2010b). Trichothecene mycotoxins like DON were suggested to serve as aggressiveness factors especially in wheat and potentially also in maize and, thus, might even promote fungal growth and ear rot symptoms (Harris et al., 1999; Maier et al., 2006). The role of zearalenone on the other hand is less clear but is also considered as a result of fungal growth. Cultivation of maize varieties resistant to GER will therefore reduce the risk of accumulation of mycotoxins.

The QTL analyses for resistance to GER and mycotoxin contamination revealed many colocalized QTL for these traits, as expected from the strong genotypic correlations (Martin et al., 2011; Martin et al., 2012a). In some cases QTL for reduced ZEA or DON contamination were detected, which were not colocalized with GER resistance QTL. In these instances, however, the individual LOD profile curves indicated peaks for GER resistance as well, yet QTL were not detected either due to non-significance or model selection criteria. Thus, “pure mycotoxin QTL”, in other words, QTL influencing mycotoxin contamination only were not found. This gives a genomic explanation for the strong genotypic correlation among these traits and further suggests mycotoxin accumulation being a consequence of fungal growth and/or presence of pleiotropic QTL. Similar to the results of the present study, Robertson-Hoyt et al. (2006) found several common QTL for Fusarium ear rot resistance and reduced mycotoxin contamination using US germplasm.

Relevance of different modes of gene action

Resistance to GER and mycotoxin accumulation is quantitatively inherited. Although Reid et al. (1994) reported indications of a major dominant gene conferring increased resistance and Yuan et al. (2008) reported a gene encoding for a guanylyl cyclase-like protein to be associated with increased resistance (both studies with Canadian maize material), genetic improvement is achieved only slowly and usually requires intensive phenotypic evaluation of genotypes. Further, information about genes or QTL, which are stably expressed across environments or genetic backgrounds, is needed. A strong influence of different environments on the expression of detected QTL was demonstrated by Ali et al. (2005) in a Canadian recombinant inbred line population. For early maize germplasm adapted to Central Europe, there has been, apart from the present study, no report on QTL controlling resistance to GER and mycotoxin contamination and neither on inheritance of these traits. In the present study, modes of gene action for these traits were studied by QTL mapping, generation means analysis and comparison of the performance of DH lines and their testcrosses.

The use of homozygous DH lines enabled the study of additive and additive-by-additive interaction effects of QTL, and the use of four test environments allowed testing for QTL-by-environment interactions (Martin et al., 2011; Martin et al. 2012a). For resistance to GER, DON and ZEA contamination, QTL-by-environment interactions were less pronounced than expected from the significant estimates of σ^2_{GE}, probably a consequence of using adjusted entry means across environments for the analyses. Additive gene action was by far more important than digenic interactions of QTL. This was indicated by the higher number of QTL having significant additive effects, their relative contributions to the total genotypic variance explained and the magnitude of their effects. In accordance with this result, the trait distributions of the DH line populations followed normal distributions and had means not significantly different from the corresponding parent lines means. Further, only in population UH007×UH006, two epistatic interactions of QTL were significant for GER resistance and one was significant for reduced DON concentration. Among these interactions of QTL, only one of the involved QTL in each interaction had also a significant and relatively strong additive effect alone. A minor role of epistasis compared to additive gene action was corroborated by generation means analysis using the five F_1-crosses, from which the DH populations had been derived, the F_2- and the first backcross generations to the parents (BC_1-P_1, BC_1-P_2) as well as the two

parent lines of each cross (Martin et al., 2012c). These analyses indicated significance of digenic epistasis only for DON contamination in one cross, namely dominance-by-dominance interaction in UH009×UH006.

From the results of the generation means analysis (Martin et al., 2012c) and from the testcross experiment (Martin et al., 2012b), there were indications of partial dominance being of importance for the expression of resistance. In generation means analysis, dominance effects were significant in some of the crosses analyzed and the flint×flint F_1-crosses were more resistant than expected from the theoretical mid-parent performance. Similarly, in the testcross experiment, flint×dent crosses were on the average significantly more resistant than expected from the theoretical mid-parent performance of the tester and corresponding DH lines. Thus, there were indications of mid-parent heterosis, which is likely to be caused by dominant gene action. Interestingly, the mid-parent and flint×flint F_1 performances in generation means analysis were stronger correlated than the mid-parent and flint×dent testcross performances in the testcross experiment. This suggested a greater role of additive effects in the intrapool crosses (flint×flint) than in the interpool crosses (flint×dent). Possibly, different resistance alleles have become fixed in the selection history of each heterotic pool and by recombining divergent lines from both pools, new allele combinations lead to an increased mean resistance. Consequently, resistance of these crosses is not easily predictable from the performance of the parents and, thus, intensive field testing of testcrosses is needed to identify the best combinations. This is in agreement with findings of Kovács et al. (1994), who observed an on the average better resistance of hybrids compared to their inbred parents and concluded that prediction of hybrid resistance was only partially possible.

Besides dominant gene action, superior performance of hybrids may also be due to increased vigor, which is known to positively contribute to increased tolerance to biotic and abiotic stresses. This is similar to what is usually observed for grain yield caused by higher genetic divergence of the parents and consequently higher heterosis especially in interpool hybrids (Melchinger and Gumber, 1998). In accordance with these observations, increased vigor of hybrids has been associated previously with increased resistance to Fusarium ear rot caused by *F. verticillioides* and *F. proliferatum* in experiments conducted in the US Cornbelt (Eller et al., 2008b).

Effects of resistance QTL in different genetic backgrounds

A commonly encountered problem in QTL mapping studies is low transferability of results from separate experiments. In the present study, QTL analyses were conducted in populations connected by common parent lines to take this issue into account. Joint linkage analysis using a common linkage map as proposed by Blanc et al. (2006) is a preferable approach in designs with connected populations, to draw inferences about consistent QTL effects across populations. However, due to shortage of common polymorphic markers among the mapping populations, QTL locations were compared using the concept of chromosomal bins (Gardiner et al., 1993).

One apparently common QTL with a large additive effect was detected in populations UH007×UH006 (*qger2, qdon2, qzea3*) and UH009×UH006 (*qger10, qdon10*) in bin 2.04, which influenced GER and mycotoxin accumulation (Martin et al., 2011; Martin et al., 2012a). Furthermore, its substantial contribution to the genotypic variance in both populations and the good marker coverage in this region suggest selecting for this QTL in a marker assisted-breeding program. In addition, it would be interesting to study this QTL under natural infection conditions, when GER severity does usually not reach the levels of infection as in the present study. The QTL had on the average an additive effect of 7.5% reduction of ear rot under artificial inoculation. It would be interesting to know, if this effect is absolute, i.e., constant under natural GER severities, which are usually lower than under artificial inoculation.

Among the 94 testcrosses, 52 carried this QTL and had either one allele of UH006, UH007 or UH009 (26 testcrosses from population UH007×UH006 and 26 from population UH009×UH006). However, in contrast to the corresponding DH lines the three allelic groups of testcrosses were not significantly different from each other for GER severity and DON concentration (Figure 3). This may indicate that this QTL acts in a recessive manner in flint×dent interpool crosses, which could partly explain the weak genotypic correlations between line and testcross performance. However, due to the low number of individuals and the presence of further segregating QTL in each of the three groups, conclusions about the mode of gene action of this QTL are preliminary. Future research on the effects of the most promising QTL detected in the present study in hybrids would be very valuable.

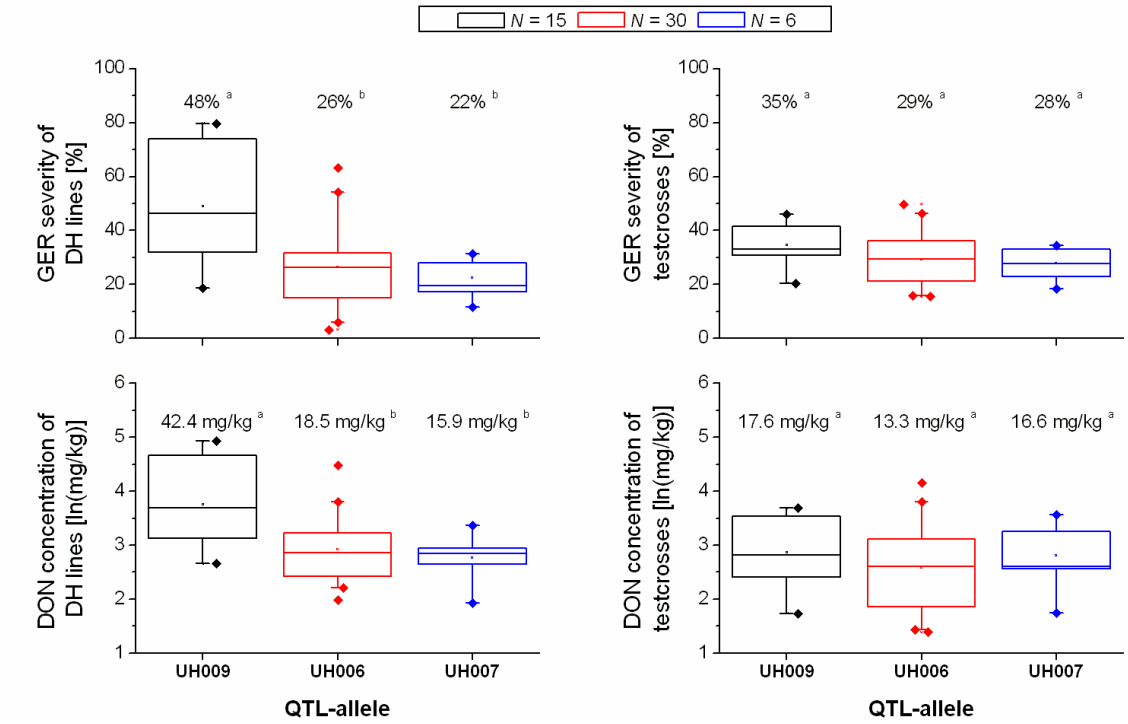

Figure 3 Boxplots of adjusted means across four environments of 51 doubled haploid lines and their testcrosses from two populations (UH007×UH006, UH009×UH006) carrying different alleles (UH006, UH007 or UH009) of a common QTL on chromosome 2 (bin 2.04) for resistance to Gibberella ear rot (GER) and deoxynivalenol (DON) contamination. Means followed by the same letter are not significantly different for a given trait at $P \leq 0.05$. *N* indicates the number of individuals in each allelic group.

Another promising marker region was found at the distal end of the long arm of chromosome 2. Detection of a QTL for GER resistance (*qger3*) and LOD peaks for reduced DON and ZEA contamination in UH007×UH006 in bin 2.08 as well as detection of two QTL for GER resistance and reduced DON contamination (*qger7*, *qdon5*) in D152×UH007 in bin 2.09 linked to a common SSR marker strongly suggested presence of a common resistance QTL across both populations (Martin et al., 2012a). In addition, there are indications for a QTL in this region contributing to unspecific resistance to further ear rot pathogens and associated mycotoxins as recently described by Xiang et al. (2010). These authors revealed common QTL regions across a range of genetic backgrounds for resistance to *Fusarium* spp. and *Aspergillus flavus*, an important maize ear pathogen causing contamination with aflatoxins. This would additionally provide an explanation for the positive correlations reported for these ear rots and their toxins (Presello et al., 2006; Robertson-Hoyt et al., 2007; Bolduan et al., 2009a; Löffler et al., 2010b).

Generally, common QTL locations across populations were relatively rarely and less often detected than expected taking into account that the populations share common parents (Martin et al., 2012a). Possible reasons for this outcome include (i) limited power of QTL detection, (ii) fixation of QTL in some of the crosses, (iii) lack of common markers, (iv) epistasis as well as (v) QTL-by-environment interactions. Possible strategies for further research in this area could include an increase of the number of mapping populations possibly compensated by a reduction of individual population sizes and/or crossing one superior line like UH006 to several diverging lines. The latter approach is known as nested association mapping and has already been demonstrated to be promising for the improvement of other resistances (McMullen et al., 2009; Kump et al., 2011; Poland et al., 2011). Genotypes carrying favorable allele combinations could then be extracted and further used for example within marker-assisted recurrent selection to increase the overall resistance level in the breeding populations.

Relationship between resistance and agronomic traits

In applied breeding programs, selection is always conducted for several traits simultaneously. Selection progress in breeding for multiple traits depends to a large extent on the magnitude of the correlations among the target traits and on, whether the correlations are favorable. Therefore, it is of crucial importance to consider the correlated response of selection for resistance on other economically important traits.

In the DH line populations, the number of days to silking was negatively correlated with GER severity, DON and ZEA concentration and this relationship was partly also reflected by the QTL mapping results (Martin et al., 2011; Martin et al. 2012a). In ten instances, QTL for DTS were colocalized with QTL for GER resistance and/or reduced DON contamination. This indicated an influence of the silking date and subsequent ear and kernel development on the resistance. This observation might be explained methodically and/or genetically. On the one hand, there might be a potential influence of the time period between inoculation and visual rating, for example a prolonged period of disease development in combination with weather conditions favoring fungal growth after inoculation of earlier flowering entries could intensify disease development. On the other

hand, linkage or pleiotropy of QTL, which influence female flowering, ear and kernel development as well as disease resistance, could have caused the observed correlations. Similar conclusions were also drawn by Robertson et al. (2006), who found weak correlations between Fusarium ear rot and days to silking. Consideration of flowering time for selection decisions on GER resistance is therefore essential to avoid a shift towards later maturity.

For GER, it is known that increased ear rot resistance leads to higher yields in environments with heavy infection of *Fusarium* spp. (Vigier et al., 2001; Eller et al., 2008b). In Central Europe, the main problem of infection with *F. graminearum* is mainly quality reduction by mycotoxin contamination. The incidence of ear rots and the contamination with mycotoxins is very much dependent on the prevalent weather conditions in a certain year. Therefore, agronomic performance of resistant maize hybrids in years with only low natural incidence of ear rots and low impact on yield is of crucial importance. In the testcross experiment (Martin et al., 2012b), GER severity and DON concentration were determined under artificial inoculation, while several agronomic traits were studied under non-inoculated conditions with low incidence of ear rot. Moderate genotypic correlations of both GER severity and DON concentration with dry matter content indicated that resistant hybrids may have later maturity and higher moisture content at harvest. Thus, even though late maturing hybrids may express resistance in the field, the risk of post-harvest accumulation of mycotoxins in improperly dried maize grains must be considered. The relationship between resistance and early plant vigor of testcrosses (rated at the four to five leaf stage) indicated that plants being more vigorous at an early stage of development tend to be more resistant to GER. However, this generally promising relationship was relatively weak. Furthermore, the resistance traits were genetically not correlated with grain yield as well as with the number of days to silking or plant height, two important traits genetically correlated to biomass yield (Lübberstedt et al., 1997; Méchin et al., 2001). Hence, there seems to be no direct penalty of disease resistance on agronomic performance. Therefore, selection for these breeding goals can most likely be performed simultaneously without negative side effects. Furthermore, as elite material was used in this study, the prospects are good to breed for resistance using the adapted germplasm, thus avoiding introgressions of exotic resistance sources, which often have poorer agronomic performance (Munkvold, 2003; Eller et al., 2008a).

Ways to genetically improve the resistance

Many questions have to be addressed by a maize breeder, who intends to improve resistance to GER and mycotoxin accumulation of the germplasm. In this study, some answers could be provided, which might be helpful to set up a selection program. However, answers to specific questions may vary depending on the breeding material and target environments. Hence, there is still need to conduct further research in this area. In this section some recommendations will be given, how a selection program could be set up and which issues should be considered.

Evaluation of methods to phenotypically assess the resistance

One critical issue in a resistance breeding program is the presence of sufficient levels of infection pressure of the pathogen of interest. In the case of *F. graminearum*, it was very helpful to ensure satisfying and homogenous infection pressures by performing artificial silk channel inoculation according to Reid et al. (1996). Artificial inoculation allowed very good differentiation of the genotypes under study. By comparison, ear rot severities under natural infection pressure allowed no reliable differentiation. Hence, in most cases there will be a need to conduct artificial inoculations, although this creates additional work load. In this regard, environments known to be conducive to heavy natural infection could be very valuable for the screening of genotypes due to lower labor costs.

Once, a decision on artificial inoculation has been made, one question that arises is how much amount of inoculum should be used. In previous studies using the *Fusarium* isolate from this study, the amount of inoculum was doubled in hybrids compared to lines due to different sizes of maize ears (Bolduan et al., 2010; Löffler et al., 2011). In these cases, conclusions about different resistance levels of lines and testcrosses were difficult to draw owing to confounding with varying amounts of inoculum. In contrast, one objective of the present study was to conduct sound comparisons between line *per se* and testcross performance and therefore equal amounts of inoculum were used. Nevertheless, in applied breeding programs, it may be reasonable to adjust inoculum dosage depending on various factors such as the objectives of the experiment (comparing vs. maximizing variation of

lines and testcrosses), aggressiveness of the used *Fusarium* isolate as well as on the resistance level of candidate lines and testers.

Conventional mycotoxin analyses are relatively expensive and it would be advantageous to substitute them. High estimates of r_g among GER severity, DON and ZEA concentration suggested that costly determination of this mycotoxin is not necessary, and visual assessment of GER is sufficient. This is underpinned by the magnitudes of the relative efficiencies of selection for reduced mycotoxin concentration based on visual ear rot rating, which are even larger than 100% in the case of GER vs. DON and about 100% in the case of GER vs. ZEA (Martin et al., 2011; Martin et al., 2012a). One limitation to this general statement would be, when different persons are involved in visual rating of GER severity, which may in consequence result in a potential bias. NIRS-based prediction of DON concentration according to a calibration of Bolduan et al. (2009b), which has been validated in the present study (Martin et al. 2012a), circumvents this problem and is also relatively inexpensive compared to DON determination by ELISA. However, visual GER rating is the cheapest alternative and should be given priority.

Application of marker-assisted selection

In quantitative genetics, marker-assisted selection (MAS) describes a method of integrating molecular genetics with artificial selection. Assuming equal selection intensities, pure MAS is superior to classical phenotypic selection, if the proportion of genotypic variance explained by QTL exceeds h^2 (Lande and Thompson, 1990). Based on the results of the present study, classical phenotypic selection for GER resistance and reduced mycotoxin contamination would be more effective than pure MAS, at least in the populations studied. However, classical phenotypic selection is based on expensive field trials with artificial inoculation and is laborious. MAS offers the advantages of cost-effective evaluation of a larger germplasm set and consequently a higher selection intensity. Further, MAS can be carried out in off-season winter nurseries, where phenotypic selection is not possible. These factors may lead to a significant improvement in the efficiency of MAS. The combination of classical phenotypic selection and MAS could be a promising strategy for maize breeders. For instance, preselection of the genotypes based on MAS before sowing in the normal growing season would increase overall selection intensity, and would allow completion of more than one selection cycle per year. Consequently, a rapid increase of the

genetic gain per year for the resistance traits could be realized. Although congruency of QTL across populations was less pronounced than expected, some major QTL for resistance to visible ear rot and mycotoxin accumulation being quite stable across environments were detected in at least two mapping populations. Focusing on such QTL in MAS seems most promising. Considering the decreasing costs of genotyping, it can be expected that in the near future marker data will be generated routinely for the whole genome. Thus, it will be possible to conduct MAS simultaneously for QTL underlying various traits of interest including resistance traits. Further, owing to the growing knowledge on the use of high density DNA markers to predict the genotypic performances for various quantitative traits, genome-wide selection as proposed by Meuwissen et al. (2001) looks promising for increasing the response to selection.

Improving line and hybrid resistance

The results of the present study suggest that selection for resistance will be efficient both in lines as well as in testcrosses. However, the correlated response of selection for resistance is expected to be quite low owing to the weak estimates of r_g between DH lines and their testcrosses (Martin et al., 2012b). Although selection among lines has several advantages, the results suggest conducting selection mainly based on performance of testcrosses in order to improve hybrid performance, the ultimate goal in maize breeding. Therefore, resources should be mainly allocated for evaluation of disease resistance of testcrosses and experimental hybrids.

Several questions must be addressed in testcross experiments. Among them, choice of the optimal tester(s) and allocation of resources are of crucial importance. Based on the results of the present study, the use of a moderately susceptible tester seems appropriate, because mid-parent heterosis for increased resistance was observed, and the use of a resistant tester could therefore complicate correct ranking of lines. This would be in accordance with Löffler et al. (2011), who recommended the use of a tester with (moderate) susceptibility to avoid masking effects of dominant alleles imparting resistance. Further, the use of a single cross of two similarly susceptible inbreds instead of an inbred as tester should be considered. Some practical advantages of using a single cross tester are better representation of the particular heterotic pool, increased and prolonged pollen shedding as well as higher vigor. In addition, testcross performance of a single cross tester

is confounded with only half of the specific combining ability effects compared to a line tester. Furthermore, it might be fruitful, if the tester was strong in terms of general combining ability for yield and other agronomically important traits. Resistance screening in testcrosses could thereby be conducted along with the normal assessment of agronomic performance. About six plants per plot would be sufficient in single cross hybrids to reliably determine GER resistance (Bolduan et al., 2009a), but more plants are recommended in the case of three-way cross hybrids to account for genotypic segregation. Shortly before harvest, inoculated ears could then be rated visually, but should be removed thereafter to get an unbiased measure of grain yield and other traits.

Although the focus of selection should be on hybrids, line resistance should not be neglected. Improved resistance to ear rots in lines will allow better seed qualities and quantities. Furthermore, owing to the prevalence of additive gene action, improvement of resistance in lines will in the long run also improve resistance of hybrids, although progress will be slow. Additive gene action further suggests that a good resistance level has to be ensured in lines of both heterotic pools (flint and dent) to obtain superior hybrids. One may focus on selection of genotypes carrying favorable marker alleles at major QTL by applying MAS in lines, as well as on elimination of very susceptible lines by phenotypic evaluations. The latter are recognized usually early and with minor testing efforts, in other words with a lower number of replications and optionally environments. Such a strategy could be implemented relatively easy in a breeding program using DH lines (Martin et al., 2012b).

Conclusions

A large number of colocalized QTL for GER resistance and reduced mycotoxin accumulation found in the present study provide a good explanation for the commonly observed strong genotypic correlations among these traits. The strong correlations corroborated earlier reports that costly determinations of mycotoxins are not necessary. Some of the detected QTL were quite large and appear suitable for MAS. The study indicated that the prospects are good to improve the resistance of the flint germplasm by utilizing promising breeding lines like UH006 and UH007 or transgressive segregants

derived from their cross thereby taking advantage of the prevalent additive mode of gene action in flints. But further research should focus on (1) fine mapping to validate the reported QTL effects and (2) the expression of the detected QTL in different genetic backgrounds, including dent×flint hybrids with regard to the final goal of breeding superior maize hybrids. The results from the present study indicated that the effects of promising resistance QTL in different backgrounds, including combinations with individuals of the opposite heterotic pool, are rather low. Therefore, it seems reasonable to allocate more resources to experiments with testcrosses than lines. In the near future, genomic tools may be available and should allow a greater efficiency of molecular breeding. Finally, our results based on elite material showed that selection of hybrids with increased agronomic performance will have no direct penalty on resistance and *vice versa* and should therefore be conducted simultaneously.

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7. Summary

Maize (*Zea mays* L.) is affected by a number of diseases. Much attention is paid to ear rot diseases, which affect grain yield and particularly grain quality. Among the various ear rots of maize, Gibberella ear rot (GER) caused by *Fusarium graminearum* Schwabe (teleomorph *Gibberella zeae* (Schwein.) Petch) is prevalent in Central Europe. This fungal pathogen produces secondary metabolites (mycotoxins), which adversely affect the health of humans and animals. Two important mycotoxins are the immunosuppressant deoxynivalenol (DON) and the mycoestrogen zearalenone (ZEA). The most efficient method to reduce mycotoxin contamination in maize is cultivation of resistant varieties. However, resistance breeding using classical phenotypic selection is laborious and time-consuming. Therefore, marker-assisted selection (MAS) may be a promising alternative to classical selection. Furthermore, for setting up a breeding program, knowledge about the relevance of the different modes of gene action and genotypic correlations among resistance and agronomic traits is required.

The objectives of this study were to (1) estimate quantitative genetic parameters for GER severity and mycotoxin concentration in connected populations of doubled haploid (DH) lines, (2) map quantitative trait loci (QTL) for GER resistance and reduced mycotoxin contamination in these populations, (3) examine the congruency of QTL in these populations, (4) evaluate the prospects of using MAS to breed for GER resistance and reduced mycotoxin contamination, (5) estimate the genotypic correlation between the resistance of DH lines *per se* and the resistance of their testcrosses, (6) evaluate the influence of selection for increased resistance on agronomic performance of hybrids and (7) examine the relevance of different modes of gene action involved in the expression of the resistance in flint maize.

Three field experiments were conducted, each of which comprised a different set of plant material. Experiment I comprised five DH line populations derived from the following F_1 crosses among elite flint inbred lines: D152×UH006, D152×UH007, UH007×UH006, UH009×UH006 and UH009×UH007. Experiment II comprised testcross progenies of 94 DH lines and a dent single cross tester. Experiment III comprised the five F_1 crosses, from which the DH populations had been derived, the F_2 and the first backcross

generations to the parents (BC_1-P_1, BC_1-P_2) as well as the two parent lines of each cross. Each experiment was conducted in four environments (year-location combinations).

Plants were artificially infected with spores of *F. graminearum* shortly after mid-silking using the silk channel inoculation technique. In all experiments, GER severity was visually rated based on the ear surface covered with mycelium in percent and DON concentration was determined. In Experiment I, ZEA concentration was additionally determined in population UH007×UH006. In Experiment II, performance of testcrosses was additionally assessed for early plant vigor, plant height, dry matter content and grain yield measured under non-inoculated conditions.

The DH lines were genotyped with 363 publicly available simple sequence repeat (SSR) DNA markers. The genetic linkage maps comprised 98 to 133 SSR markers, depending on the population. QTL analyses were performed for resistance to GER, DON and ZEA contamination.

Estimates of genotypic and genotype-by-environment interaction variances in Experiment I for GER severity and mycotoxin concentration were significant and heritabilities were moderately high to high in all populations. Thus, differences among DH lines for the resistance traits were mainly caused genetically and the resistance response varied depending on the environment. Owing to the effectiveness of artificial inoculation, the prospects are good to improve line resistance using a small number of test environments.

QTL were detected in the four largest populations (D152×UH007, UH007×UH006, UH009×UH006 and UH009×UH007). Depending on the population, the mapped QTL together explained 21-51% of the genotypic variance for GER severity and 19-45% for DON concentration and 52% for ZEA concentration. Additive gene action was more important than digenic interactions of QTL, as indicated by the number of QTL having significant additive effects, their relative contributions to the total genotypic variance explained and the magnitude of their effects.

Colocalized QTL for resistance to GER and mycotoxin contamination were identified in each mapping population. This was in agreement with strong genotypic correlations among these traits. QTL located at similar positions were detected across three populations in two chromosomal regions and across two populations in additional two

regions. The results of this study indicated that a combination of classical phenotypic selection and MAS is a promising strategy for resistance breeding.

In Experiment II, significant genotypic variation for resistance in lines and testcrosses showed that selection will be successful in both groups. Owing to low genotypic correlations between lines and testcrosses, however, resources should be mainly allocated to the evaluation of GER in testcrosses. Correlations of resistance with agronomic traits were weak or not significant. Therefore, selection for resistance and better agronomic performance can be carried out simultaneously.

In Experiment III, generation means analysis indicated a prevalence of additive gene action for resistance. Significant dominance effects were found in only one cross for resistance to GER, but in four crosses for resistance to DON contamination. Owing to prevalence of additive gene action, the prospects are good to improve the resistance of the flint germplasm and to accumulate more favorable gene combinations in future breeding lines. Comparing the hybrid performance of flint×flint crosses of Experiment II and flint×dent crosses of Experiment III with their corresponding mid-parent performances indicated mid-parent heterosis for resistance. Therefore, prediction of hybrid performance based on performance of their parents will be possible only to a very limited extent.

Future research should focus on fine mapping and validating of the detected QTL. For an efficient use of the QTL in a marker-assisted breeding program, knowledge about their effects in different genetic backgrounds is needed. Of particular importance are thereby the QTL effects in flint×dent crosses, which represent the preferred type of hybrid in Central European maize breeding programs.

8. Zusammenfassung

Mais (*Zea mays* L.) wird von zahlreichen Krankheiten befallen. Große Aufmerksamkeit wird dabei den Kolbenfäulen zu Teil, welche den Kornertrag und besonders die Kornqualität beeinträchtigen. Kolbenfusariosen sind die wichtigsten Kolbenfäulen an Mais. In Mitteleuropa ist *Fusarium graminearum* Schwabe (teleomorph *Gibberella zeae* (Schwein.) Petch) der wichtigste Erreger dieser Krankheit. Dieser pilzliche Schaderreger produziert sekundäre Metabolite (Mykotoxine), die gesundheitsschädlich für Menschen und Tiere sind. Zwei wichtige Mykotoxine sind das immunsuppressiv wirkende Deoxynivalenol (DON) und das Mykoöstrogen Zearalenon (ZEA). Der effizienteste Weg Mykotoxinkontaminationen zu vermeiden ist der Anbau resistenter Sorten. Allerdings ist die Resistenzzüchtung mittels klassischer phänotypischer Selektion arbeits- und zeitaufwendig. Daher könnte die Marker-gestützte Selektion eine vielversprechende Alternative zur klassischen Selektion darstellen. Darüber hinaus sind für die Planung eines Zuchtprogrammes Kenntnisse über die Bedeutung verschiedener Genwirkungsweisen und über genotypische Korrelationen zwischen der Resistenz und agronomischen Merkmalen notwendig.

Die Ziele der vorliegenden Arbeit waren, (1) quantitativ-genetische Parameter für Kolbenfusariumbefall und Mykotoxinkonzentration in verbundenen Populationen doppelt-haploider (DH) Linien zu schätzen, (2) sog. *quantitative trait loci* (QTL) für Fusariumresistenz und reduzierte Mykotoxinkontamination in diesen Populationen zu kartieren, (3) die Übereinstimmung der QTL in diesen Populationen zu untersuchen, (4) die Aussichten der Marker-gestützten Selektion für die Züchtung auf Fusariumresistenz und reduzierte Mykotoxinkontamination zu bewerten, (5) die genotypische Korrelation zwischen der Resistenz von DH-Linien *per se* und deren Testkreuzungen zu schätzen, (6) die Auswirkung der Selektion auf verbesserte Resistenz auf agronomische Eigenschaften von Hybriden zu bewerten und (7) die Bedeutung verschiedener Genwirkungsweisen auf die Expression der Resistenz in Flint-Mais zu untersuchen.

Drei Feldexperimente wurden durchgeführt, in denen jeweils unterschiedliches Pflanzenmaterial geprüft wurde. In Experiment I wurden fünf DH-Populationen untersucht, die aus folgenden F_1-Kreuzungen von Elite-Flint-Inzuchtlinien erstellt wurden: D152×UH006, D152×UH007, UH007×UH006, UH009×UH006 und UH009×UH007. In

Experiment II wurden die Testkreuzungsnachkommen von 94 DH-Linien und einer Dent-Einfachkreuzung, die als Tester fungierte, untersucht. In Experiment III wurden neben den fünf F_1-Kreuzungen, aus denen die DH-Populationen abgeleitet wurden, deren F_2- und erste Rückkreuzungsgenerationen zu beiden Eltern (BC_1-P_1, BC_1-P_2) sowie die zwei Elternlinien jeder Kreuzung untersucht. Jedes Experiment wurde in vier Umwelten (Jahr-Ort-Kombinationen) durchgeführt.

Die Versuchspflanzen wurden kurz nach dem Erscheinen der Narbenfäden künstlich mit Sporen von *F. graminearum* infiziert. In allen Experimenten wurde der Kolbenfusariumbefall visuell als prozentualer Anteil der mit Mycel überdeckten Kolbenoberfläche bonitiert und die DON-Konzentration in den Körnern gemessen. In Experiment I wurde zusätzlich in der Population UH007×UH006 die ZEA-Konzentration erfasst. In Experiment II wurden in den Testkreuzungen zusätzlich Jugendentwicklung, Wuchshöhe, Trockenmassegehalt und Kornertrag unter nicht-inokulierten Bedingungen bestimmt.

Die DH-Linien wurden mit 363 öffentlich zugänglichen sog. *simple sequence repeat* (SSR) DNA-Markern genotypisiert. Die genetischen Kopplungskarten enthielten je nach Population zwischen 98 und 133 SSR-Marker.

Die Schätzwerte für die genotypischen Varianzen und Genotyp-Umwelt-Wechselwirkungsvarianzen für Kolbenfusariumbefall und Mykotoxinkonzentrationen waren in Experiment I in allen Populationen signifikant und die Heritabilitäten waren mäßig hoch bis hoch. Somit waren die Unterschiede zwischen den DH-Linien hauptsächlich genetisch bedingt. Die Resistenzreaktion wurde jedoch auch von der Umwelt beeinflusst. Aufgrund der Effizienz künstlicher Inokulation sind die Aussichten günstig, die Linienresistenz mit einer geringen Anzahl an Test-Umwelten zu verbessern.

QTL wurden in den vier größeren Populationen gefunden (D152×UH007, UH007×UH006, UH009×UH006 und UH009×UH007). Je nach Population erklärten die kartierten QTL zusammen 21-51% der genotypischen Varianz für Kolbenfusariumbefall, 19-45% für DON-Konzentration und 52% für ZEA-Konzentration. Additive Genwirkung war von größerer Bedeutung als digenische Wechselwirkungen von QTL, was aufgrund der Anzahl an QTL mit signifikanten additiven Effekten, deren relativen Beiträgen zur gesamten erklärten genotypischen Varianz und der Größe ihrer Effekte ersichtlich war.

In Übereinstimmung mit den engen genotypischen Korrelationen wurden kolokalisierte QTL für die Resistenz gegen Kolbenfusarium und Mykotoxinkontamination in jeder Kartierungspopulation gefunden. QTL an ähnlichen Positionen wurden auf zwei Chromosomenabschnitten über drei Populationen hinweg und auf weiteren zwei Chromosomenabschnitten über zwei Populationen hinweg gefunden. Die Ergebnisse deuteten darauf hin, dass eine Kombination von klassischer phänotypischer und Marker-gestützter Selektion eine vielversprechende Strategie für die Resistenzzüchtung ist.

In Experiment II waren die genotypischen Varianzen für die Resistenz in den Linien und Testkreuzungen signifikant. Dies zeigt, dass in beiden Gruppen erfolgreich selektiert werden kann. Jedoch sollten aufgrund der geringen genotypischen Korrelationen zwischen Linien und Testkreuzungen die vorhandenen Ressourcen größtenteils zur Prüfung von Testkreuzungen verwendet werden. Die Korrelationen zwischen der Resistenz und den agronomischen Merkmalen waren schwach oder nicht signifikant. Dadurch kann problemlos auf Resistenz und verbesserte agronomische Eigenschaften selektiert werden.

In Experiment III zeigten Generationsmittelwertanalysen eine vornehmliche Bedeutung additiver Genwirkung auf die Resistenz. Signifikante Dominanzeffekte wurden nur in einer Kreuzung für die Resistenz gegen Kolbenfusarium gefunden, allerdings in vier Kreuzungen für Resistenz gegen DON-Kontamination. Aufgrund der Bedeutung der additiven Genwirkungsweise sind die Aussichten gut, die Resistenz im Flint-Material zu verbessern und günstige Genkombinationen in zukünftigen Zuchtlinien anzureichern. Vergleiche der Flint×Flint-Hybridkreuzungen aus Experiment II und der Flint×Dent-Hybridkreuzungen aus Experiment III mit den jeweiligen Elternmittelwerten deuteten auf eine Überlegenheit der Nachkommen gegenüber dem Elternmittel aufgrund von Heterosis hin. Aufgrund dessen wird es nur eingeschränkt möglich sein, die Resistenz von Hybriden aufgrund der Resistenz ihrer Eltern vorherzusagen.

Zukünftige Forschungsarbeiten sollten sich auf die Feinkartierung und Validierung der gefundenen QTL konzentrieren. Um die QTL effizient zu nutzen, sind Kenntnisse über deren Effekte in unterschiedlichen genetischen Hintergründen notwendig. Dabei sind die QTL-Effekte in Flint×Dent-Hybridkreuzungen von besonderer Bedeutung, da diese den bevorzugten Hybrid-Typ in Mitteleuropäischen Maiszüchtungsprogrammen darstellen.

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10. Curriculum vitae

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11. Erklärung

Hiermit erkläre ich an Eides statt, dass die vorliegende Arbeit von mir selbst verfasst wurde und lediglich unter Zuhilfenahme der angegebenen Quellen und Hilfsmittel angefertigt wurde. Wörtlich oder inhaltlich übernommene Stellen wurden als solche gekennzeichnet.

Die vorliegende Arbeit wurde in gleicher oder ähnlicher Form noch keiner anderen Institution oder Prüfungsbehörde vorgelegt.

Insbesondere erkläre ich, dass ich nicht früher oder gleichzeitig einen Antrag auf Eröffnung eines Promotionsverfahrens unter Vorlage der hier eingereichten Dissertation gestellt habe.

_____

Matthias Martin

Hohenheim, 27.07.2011